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ORIGINAL ARTICLE

Molecular and synaptic organization of GABA_A receptors in the cerebellum: Effects of targeted subunit gene deletions

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Abstract

GABA_A receptors form heteromeric GABA-gated chloride channels assembled from a large family of subunit genes. In cerebellum, distinct GABA_A receptor subtypes, differing in subunit composition, are segregated between cell types and synaptic circuits. The cerebellum therefore represents a useful system to investigate the significance of GABA_A receptor heterogeneity. For instance, studies of mice carrying targeted deletion of major GABA_A receptor subunit genes revealed the role of α subunit variants for receptor assembly, synaptic targeting, and functional properties. In addition, these studies unraveled mandatory association between certain subunits and demonstrated distinct pharmacology of receptors mediating phasic and tonic inhibition. Although some of these mutants have a profound loss of GABA_A receptors, they exhibit only minor impairment of motor function, suggesting activation of compensatory mechanisms to preserve inhibitory networks in the cerebellum. These adaptations include an altered balance between phasic and tonic inhibition, activation of voltage-independent K⁺ conductances, and upregulation of GABA_A receptors in interneurons that are not affected directly by the mutation. Deletion of the $\alpha 1$ subunit gene leads to complete loss of GABA_A receptors in Purkinje cells. A striking alteration occurs in these mice, whereby presynaptic GABAergic terminals are preserved in the molecular layer but make heterologous synapses with spines, characterized by a glutamatergic-like postsynaptic density. During development of $\alpha 1^{0/0}$ mice, GABAergic synapses are initially formed but are replaced upon spine maturation. These findings suggest that functional GABA_A receptors are required for long-term maintenance of GABAergic synapses in Purkinje cells.

Key words: Knockout mice, benzodiazepine, Purkinje cell, granule cell, GABAergic synapse, interneurons

Introduction

Owing to its apparent simplicity and stereotyped organization, with a clear segregation of cell types in distinct layers, the cerebellum represents an excellent structure to investigate the anatomical and functional organization of neuronal networks and major neurotransmitter systems. Most synaptic inhibition in the cerebellum is mediated by GABA_A receptors, which are highly abundant despite the overwhelming presence of glutamatergic synapses formed by parallel fibers on Purkinje cell spines. GABA_A receptors belong to the superfamily of ligand-gated ion channels, along with nicotinic acetylcholine receptors, glycine receptors and 5-HT₃ receptors (1). They form heteropentameric chloride channels gated by GABA and modulated by several clinically relevant drugs, including benzodiazepines, barbiturates, neurosteroids, and ethanol (2–4). They are assembled for a large family of subunits ($\alpha 1$ –6, $\beta 1$ –3, $\gamma 1$ –3, δ , ϵ , π , and θ in mammalian brain), of

which 13 are expressed in the cerebellum (5). Accordingly, the molecular organization of GABA_A receptors is only partially understood and the functional significance of this diversity in cerebellar inhibitory circuits is slowly starting to emerge.

Targeted deletion of GABA_A receptor subunit genes by homologous recombination has contributed much to our understanding of GABA_A receptors. Focusing on the cerebellum, numerous studies have investigated molecular, pharmacological and functional properties of GABA_A receptors that are less amenable for analysis in other brain regions (6). The present review gives a broad overview of recent reports on the molecular and synaptic organization of GABA_A receptors in cerebellar cortex. It highlights major findings from knockout mice, with a particular focus on compensatory mechanisms allowing preservation of network organization and function despite the loss of a major constituent of synaptic transmission.

Organization of GABA_A receptors in the cerebellar cortex

The heterogeneity of GABA_A receptors in the cerebellar cortex stands in striking contrast to the apparent simplicity of GABAergic circuits, which are formed by four types of interneurons (stellate and basket cells in the molecular layer, Golgi cells and Lugaro cells in the granule cell layer), in addition to Purkinje cells (PC). Each type of interneuron is activated by granule cells (parallel fiber synapses) and innervates specific targets, including other interneurons (Figure 1) (7). In total, 13 GABA_A receptor subunits have been detected immunochemically, including $\alpha 1$ – $\alpha 6$, $\beta 1$ – $\beta 3$, $\gamma 1$ – $\gamma 3$, and δ (5). The cellular distribution pattern has not yet been established for all these subunits but the major subunit combinations comprise $\alpha 1\beta\gamma 2$, $\alpha 6\beta\gamma 2$, ($\alpha 1$) $\alpha 6\beta\gamma\delta$, $\alpha 3\beta\gamma 2$, as well as $\alpha 2\beta\gamma 1$ (x indicates that the β subunit variants have not been determined). Despite this plethora of subunits, the majority of cell types express a limited repertoire of GABA_A receptor subtypes (Figure 1). For example, PC express exclusively $\alpha 1\beta\gamma 2$ -GABA_A receptors; stellate/basket cells, $\alpha 1\beta\gamma 2$ and $\alpha 3\beta\gamma 2$; Golgi cells $\alpha 3\beta\gamma 2$; Bergmann glia, $\alpha 2/\beta\gamma 1$ (8–14). The notable exception comes from granule cells, which express multiple receptor subtypes, despite receiving GABAergic input only from Golgi cells (15). Most

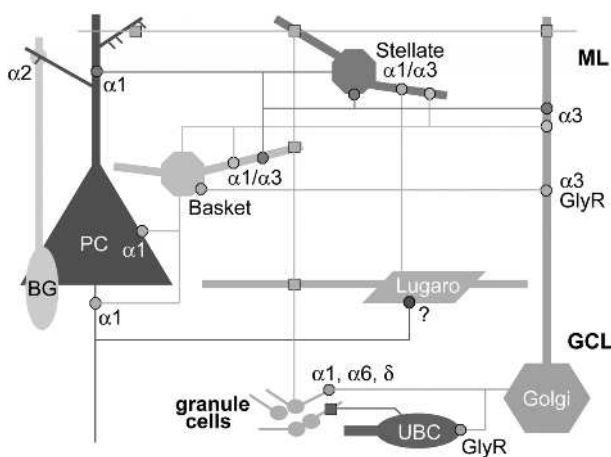


Figure 1. Schematic organization of GABAergic circuits and GABA_A receptors in the cerebellar cortex. Each cell type is indicated with a different color. Dendrites are shown in thick lines and axons in thin lines. GABAergic synapses (octagons) are shown with the color corresponding to the presynaptic cell and the main GABA_A receptor subtype(s) or glycine receptors (GlyR) present postsynaptically are indicated. Glutamatergic synapses are denoted with squares. Note that Lugaro cells innervate all cell types in the molecular layer except PC. In turn, the only inhibitory input they receive comes from PC. It is not known which GABA_A receptor subtype(s) they express. Basket/stellate cells express both $\alpha 1$ - and $\alpha 3$ -GABA_A receptor, but it is not established whether they are segregated between afferents from other basket/stellate cells and from Lugaro cells. Bergman glial cells (BG) express an unusual GABA_A receptor subtype, containing the $\alpha 2$ and $\gamma 1$ subunit, which is enriched at sites of contact with PC. Abbreviations: GCL, granule cell layer; ML, molecular layer; UBC, unipolar brush cell.

of these receptors are assembled from various combinations of $\alpha 1$, $\alpha 6$, $\beta 2$, $\beta 3$, $\gamma 2$ and δ subunit (8,12,13,16). No information is available for Lugaro cells and unipolar brush cells (17), and conversely, several subunits expressed at low levels ($\alpha 4$, $\alpha 5$, $\gamma 3$) have been identified by Western blotting only (5,18).

Depending on the cell type and subunit combination, GABA_A receptors can be located postsynaptically or extrasynaptically, where they mediate phasic and tonic inhibition, respectively (19). Postsynaptic GABA_A receptors in cerebellar neurons largely are co-localized with gephyrin, a cytoplasmic protein that is exclusively present in symmetric synapses (12). By immunofluorescence staining, they are recognized as bright clusters distributed on dendrites and somata, as well as on glomeruli of the granule cell layer (see Figure 3). Using this approach, the synaptic localization of $\alpha 1$ - and $\alpha 3$ -GABA_A receptors was shown in the molecular layer (12); in glomeruli, $\alpha 1$ - and in part $\alpha 6$ -GABA_A receptors can also be detected postsynaptically, confirming ultrastructural studies using postembedding immunogold electron microscopy (20–22). In whole cell patch clamp recordings of PC, spontaneous IPSCs of large amplitude predominate over EPSCs, indicating that PC are under powerful control from stellate and basket cells (23). These synaptic currents are mediated by GABA_A receptors with no evidence for a contribution by strychnine-sensitive glycine receptors (24). In addition, PC also carry extrasynaptic GABA_A receptors diffusely distributed on the cell surface and are modulated by tonic inhibition, notably due to GABA release from basket cells (25). Similar, high frequency and high amplitude sIPSCs have been well characterized also in stellate cells (26,27), underscoring the major role played by postsynaptic GABA_A receptors in the molecular layer.

In granule cells, a major fraction of GABA_A receptors is associated with the δ subunit (29,30), forming extrasynaptic receptors with a high affinity for GABA (31,32) and diffusely distributed on the soma and dendrites (12,22,28). The presence of the δ subunit, which does not co-assemble with the $\gamma 2$ subunit, is determinant for the location of GABA_A receptors relative to postsynaptic sites and for their functional properties. Electrophysiologically, tonic inhibition in granule cells is independent of Golgi cell action potentials (33,34). Rather, it is produced mainly by non-vesicular release of GABA and is modulated by type 1 and type 3 GABA transporters (33). So far, it is not known whether this complex regulation of tonic inhibition is related to the heterogeneity of extrasynaptic GABA_A receptors in granule cells. However, it should be emphasized that the role of the δ subunit is forming receptors mediating tonic inhibition is not restricted to the cerebellum, but also occurs in thalamus and hippocampus (35,36). Furthermore, these receptors

are a major target for neurosteroids, which reduce neuronal excitability by enhancing tonic inhibition mediated by δ -GABA_A receptors (37).

A distinct population of 'extrasynaptic', diazepam-insensitive GABA_A receptors, with a probably unique subunit composition in brain ($\alpha 2\beta\gamma 1$), is found in Bergman glia cells (10,11,14). These receptors are most abundant during early postnatal maturation and modulate K⁺ conductances that are transiently expressed by immature Bergmann glia. As shown by immunoelectron microscopy in adult brain (10), GABA_A receptors are most concentrated in glial processes wrapping PC somata and dendrites, in particular in the vicinity of symmetric synapses formed by basket cells. However, they are also concentrated in processes in contact with PC spines, in which GABA_A receptors are occasionally found. This highly regulated location suggested a role for sensing GABAergic synaptic function in PC (10).

Synaptic inhibition in the cerebellum is also mediated, to a lesser extent, by glycine receptors (38,39). A majority of Golgi and Lugaro cells have a dual GABAergic/glycinergic phenotype (40,41). The specificity of transmission in their postsynaptic targets is ensured by the differential expression of receptors. Thus, spontaneous IPSCs in granule cells are mediated selectively by GABA_A receptors (39), whereas Golgi cells, Lugaro cells, unipolar brush cells, and possibly other interneurons exhibit either glycinergic or mixed glycinergic/GABAergic currents (Figure 1) (42,43). At least in Golgi cells, GABA_A and glycine receptors can co-exist within a given postsynaptic site (42).

A distinctive feature of postsynaptic GABA_A receptors in the molecular layer is their colocalization with the dystrophin-glycoprotein complex (DGC), including β -dystroglycan, dystrophin, and α -/ β -dystrobrevin (44–47). The DGC is required for proper maturation or maintenance of postsynaptic GABA_A receptors in PC, as shown by a decreased number and size of clusters in mutant mice lacking either dystrophin, or both α - and β -dystrobrevin. As a consequence, synaptic inhibitory input is reduced on PC of mice lacking dystrophin (48). While the relevance of the DGC in specific GABAergic synapses is unclear, it is required for proper expression of long-term depression in PC (49), suggesting a role in synaptic plasticity. A similar situation occurs in the cerebral cortex and hippocampal formation, where large subsets of inhibitory synapses are associated with the DGC (45). In the hippocampus, absence of the DGC leads to alterations of short- and long-term synaptic plasticity (50,51). However, the DGC cannot be essential for the formation of GABAergic synapses or clustering of GABA_A receptors, since it is absent in many other brain areas, such as thalamus, basal ganglia, tectum, etc. Furthermore, dystrophin expression occurs after

formation of GABAergic synapses during postnatal development (45).

The maturation of GABA_A receptors during ontogeny of the cerebellum has been investigated mainly in granule cells and in PC (52,53). The subunit composition and pharmacological profile of GABA_A receptor in granule cells are developmentally regulated and these changes are activity-dependent (54–57). A transient expression of the $\alpha 2$ and $\alpha 3$ subunit occurs at early postnatal stages (58–60), whereas the $\alpha 6$ and δ subunit, which are expressed selectively in granule cells (61), is delayed (11,55,60) and parallels the formation of functional GABAergic synapses from Golgi cells (62–65). This maturation pattern contrasts with that of PC, which express a prominent $\alpha 1$ subunit immunoreactivity at birth, before having formed a monolayer and being innervated by basket and stellate cells (66,67).

Effects of targeted subunit gene deletion on cerebellar GABA_A receptors

Targeted deletions of eight GABA_A receptor subunits ("full knockout" mice) have been reported so far in the literature ($\alpha 1$, $\alpha 3$, $\alpha 5$, $\alpha 6$, $\beta 2$, $\beta 3$, $\gamma 2$, δ). Among these only four have been analyzed for their effects on assembly of GABA_A receptors and function of the GABAergic system in the cerebellum ($\gamma 2$, $\alpha 6$, δ , $\alpha 1$). The major alterations reported in these studies concern the function and pharmacology of the remaining GABA_A receptors, which are partially altered due to loss of an additional subunit that has an obligatory association with the deleted subunit (for example, δ subunit in $\alpha 6^{0/0}$ mice) or due to compensatory changes in expression of remaining subunit(s) (68,69). Unexpectedly, even targeted deletions affecting major GABA_A receptor subtypes, such as $\alpha 1$ -GABA_A receptors, have only minor behavioral consequences, suggesting that widespread adjustments take place within and outside the GABAergic system to ensure preserved function.

Most $\gamma 2^{0/0}$ mice die shortly after birth and a small percentage survive up to the third postnatal week (70). In these mice, there is an almost complete loss of $\alpha 1$ subunit and gephyrin clusters in the cerebellum, in line with the requirement of the $\gamma 2$ subunit for postsynaptic aggregation of GABA_A receptors and gephyrin (71,72). No specific compensation with another $\gamma 2$ subunit variant was observed, indicating indirectly that the $\gamma 1$ and $\gamma 3$ subunits do not contribute significantly to neuronal GABA_A receptors in the mouse cerebellum.

Deletion of the $\alpha 6$ subunit gene, which is exclusively expressed in cerebellar granule cells, caused a 50% overall reduction of GABA_A receptors in the cerebellum (73), underscoring the relative abundance of the $\alpha 6$ subunit in these cells.

Unexpectedly, this mutation caused the disappearance of the δ subunit protein (74). However, since mRNA levels for the δ subunit were not affected, this finding suggested that the δ subunit protein requires the $\alpha 6$ subunit for assembly in a receptor complex and is degraded rapidly in the absence of this mandatory partner. As a consequence, deletion of a single subunit led to the loss of multiple GABA_A receptor subtypes in granule cells. This conclusion was confirmed by an autoradiographic analysis showing marked alterations of the pharmacological profile of GABA_A receptors in the granule cell layer of $\alpha 6^{0/0}$ mice, best explained by a concurrent loss of $\alpha 6\beta\gamma 2$ - and $\alpha 6\beta\gamma\delta$ -GABA_A receptors (69). No compensation by the $\alpha 1$ subunit was detected in glomeruli or in the molecular layer of $\alpha 6^{0/0}$ mice (73), indicating that the $\alpha 1$ and $\alpha 6$ subunit are regulated independently, although their genes are present in the same chromosomal cluster (75). Interestingly, while $\alpha 6^{0/0}$ mice display no major spontaneous phenotype, a clear motor deficit could be shown upon treatment with diazepam, which could be reversed by flumazenil, indicating that it was mediated by the remaining, mainly postsynaptic, GABA_A receptors in these mice (76).

Deletion of the δ subunit gene did not result in a loss of GABA_A receptors in the cerebellum, as shown by autoradiography and Western blot analysis. An increase in receptors containing the $\gamma 2$ subunit was noted, along with $\alpha 6$ and $\beta\gamma$, suggesting that $\gamma 2$ and δ compete for assembly with these subunits in granule cells from wildtype mice (77). Functionally, a loss of modulation of sIPSCs by the neurosteroid THDOC selectively occurs in granule cells, but not stellate cells of $\delta^{0/0}$ mice (78), underscoring the importance of δ -GABA_A receptors for the *in vivo* action of neurosteroids (79). Behaviorally, deletion of the δ subunit gene produces no overt impairment in motor function or motor learning, similarly to the $\alpha 6$ subunit gene (76). This observation should not lead to the conclusion that tonic inhibition mediated by extrasynaptic GABA_A receptors in granule cells is irrelevant. Rather, the lack of phenotype is due to compensatory upregulation of voltage-independent K⁺ channels (two-pore-domain K⁺ channels TASK-1 and TASK-3), which increases the membrane conductance to the same extent as the tonic inhibition mediated by $\alpha 6$ -GABA_A receptors in wildtype mice (80).

Like for the $\alpha 6$ subunit, targeted deletion of the $\alpha 1$ subunit gene has major consequences for GABA_A receptors in the cerebellum. A profound loss of binding sites for benzodiazepine ligands occurs (81). This decrease is mirrored in the 50% reduction of $\beta 2,3$ and $\gamma 2$ subunit proteins in crude membrane extracts from the cerebellum (18,81), pointing to a decrease in the assembly of GABA_A receptors in the absence of the $\alpha 1$ subunit. This phenotype is not

unique to the cerebellum but has been observed in other brain regions, as well (82). Despite the major reduction in the number of GABA_A receptors, $\alpha 1^{0/0}$ mice display no anatomical anomaly and no gross behavioral impairment (83–85). The most obvious deficits include a mild impairment of motor coordination and a kinetic and postural tremor that is reminiscent of essential tremor in human; this tremor was responsive to drug therapies that alleviate symptoms of essential tremor in patients and is likely related to the loss of $\alpha 1$ -GABA_A receptors in multiple stations of the motor system, including the cerebellum (86).

Compensatory changes in GABA_A receptor subunit expression in $\alpha 1^{0/0}$ mice

A major feature of $\alpha 1^{0/0}$ mice, despite the 50% overall loss of GABA_A receptors, is the compensatory up-regulation of subunits that are normally expressed at low levels, which might partially replace the missing $\alpha 1$ -GABA_A receptors. In particular, expression of the $\alpha 3$, $\alpha 4$ and $\alpha 6$ subunit proteins is increased, as shown by two independent *in vivo* studies (18,81). *In vitro*, the loss of $\alpha 1$ -GABA_A receptors induced an increase in $\alpha 6\beta\gamma\delta$ -receptors (and possibly $\alpha 4\beta\gamma\delta$), suggesting that tonic inhibition partially compensates for the loss of synaptic receptors (87). Functionally, these adaptations are reflected in the maintenance of postsynaptic currents in granule cells, but with altered kinetic properties. In particular, the decay time constants of remaining receptors are prolonged, reflecting the substitution of $\alpha 1$ -GABA_A receptors by another subtype. Since $\alpha 1$ -GABA_A receptors have faster kinetics than those containing the $\alpha 2$ or $\alpha 3$ subunits (88,89), this factor was taken as an index for the sustained presence of these subunits in mature granule cells of mutant mice (87,90).

In contrast to granule cells, no compensation occurs in PC which lose all functional GABA_A receptors in mutant mice. Patch clamp recording analyses showed a complete loss of spontaneous and evoked IPSCs and no compensation by strychnine-sensitive glycine receptors (9,86). No evidence for tonic inhibition could be found either in PC of $\alpha 1^{0/0}$ mice. A detailed immunohistochemical analysis was therefore undertaken to understand how this loss of GABA_A receptors affects the synaptic organization of the GABAergic system in the molecular layer (18). On the cellular level, this study confirmed the reduction of $\beta 2,3$ and $\gamma 2$ subunit expression, as well as the upregulation of the $\alpha 3$ subunit, mainly in interneurons of the molecular layer (Figure 2). Subcellularly, the loss of $\alpha 1$ subunit causes a strong, but only partial decrease of gephyrin clusters in the molecular layer (Figure 3). Staining for the $\alpha 3$ subunit, which is normally present in a minor fraction of gephyrin clusters, revealed a prominent

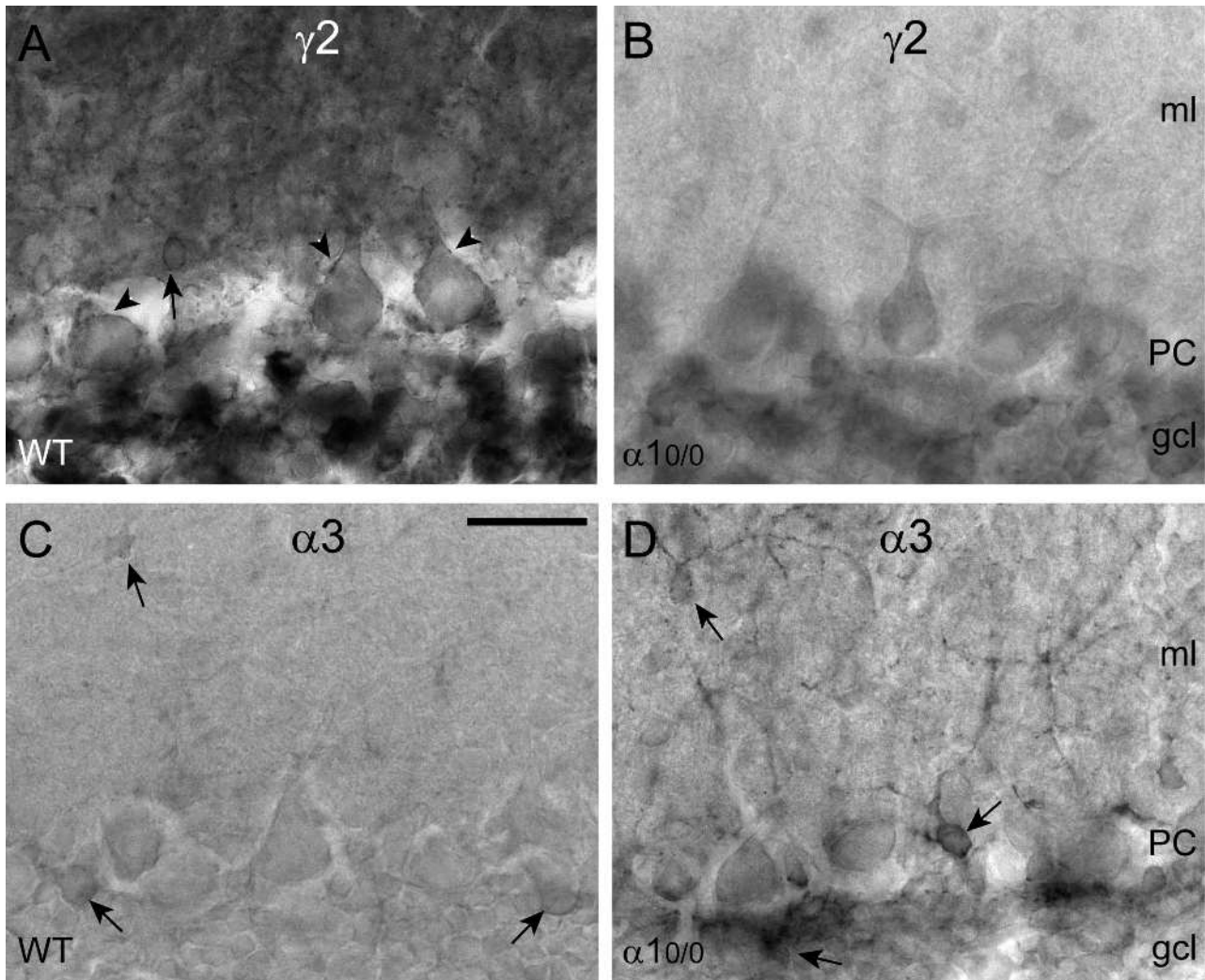


Figure 2. Differential regulation of GABA_A receptor subtypes in the cerebellar cortex of $\alpha 1^{0/0}$ mice, as shown by immunoperoxidase staining for the $\gamma 2$ and $\alpha 3$ subunit. (A) Strong and widespread expression of the $\gamma 2$ subunit in the molecular layer (ml), in PC and in the granule cell layer (gcl) of wildtype (WT) mice. The arrow points to a strongly labeled interneuron and arrowheads indicate $\gamma 2$ subunit immunoreactivity on PC, outlining the cell surface. (B) Marked reduction of $\gamma 2$ subunit staining in a section from a mutant mouse. Note that PC are unlabeled. The granule cell layer (gcl) also appears weakly labeled, and interneurons in the ml are almost undetectable. (C–D) Striking up-regulation of the $\alpha 3$ subunit, which in wildtype is weakly expressed in Golgi cells and some ml interneurons (arrows). In $\alpha 1^{0/0}$ mice, the $\alpha 3$ subunit staining outlines individual interneurons in the ml and in the gcl (arrows), whereas PC remain unlabeled. Scale bar, 50 μ m.

increase in the number of $\alpha 3$ subunit clusters in the molecular layer, most likely representing postsynaptic receptors in stellate/basket cells and Golgi cell dendrites (Figure 3). These observations are in line with a study in which miniature IPSPs recorded from stellate cells in whole-cell patch clamp experiments had slower kinetics in $\alpha 1^{0/0}$ mice than in wildtype, compatible with the expression of $\alpha 3$ -GABA_A receptors (88). The changes in $\alpha 3$ subunit expression were specific for this subunit, since no alteration of the $\alpha 2$ subunit (present in Bergman glia) was observed, whereas the $\alpha 4$ and $\alpha 5$ subunits were not detected immunohistochemically in the cerebellum of either wildtype or $\alpha 1^{0/0}$ mice (18).

The comparison of compensatory changes in GABA_A receptor expression between the granule cell layer and the molecular layer in $\alpha 1^{0/0}$ mice highlights major features of the regulation of these

receptors: (i) In granule cells expressing multiple GABA_A receptor subtypes, an increase of the remaining subunit(s) might replace the missing subunit. However, this compensation is only partial, since postsynaptic GABA_A receptors colocalized with gephyrin and mediating mainly phasic inhibition appear to be replaced by extrasynaptic receptors mediating tonic inhibition. This finding is supported by the decreased $\gamma 2$ subunit staining in the granule cell layer. A similar situation has been observed in neurons of the thalamic ventrobasal complex, in which postsynaptically clustered $\alpha 1$ -GABA_A receptors are replaced by extrasynaptic $\alpha 4$ -GABA_A receptors lacking the $\gamma 2$ subunit and gephyrin (18). Taken together, these observations suggest that GABA_A receptor subtypes are not interchangeable within a given type of neuron and that the lack of interaction of $\alpha 4$ or δ -GABA_A receptors with

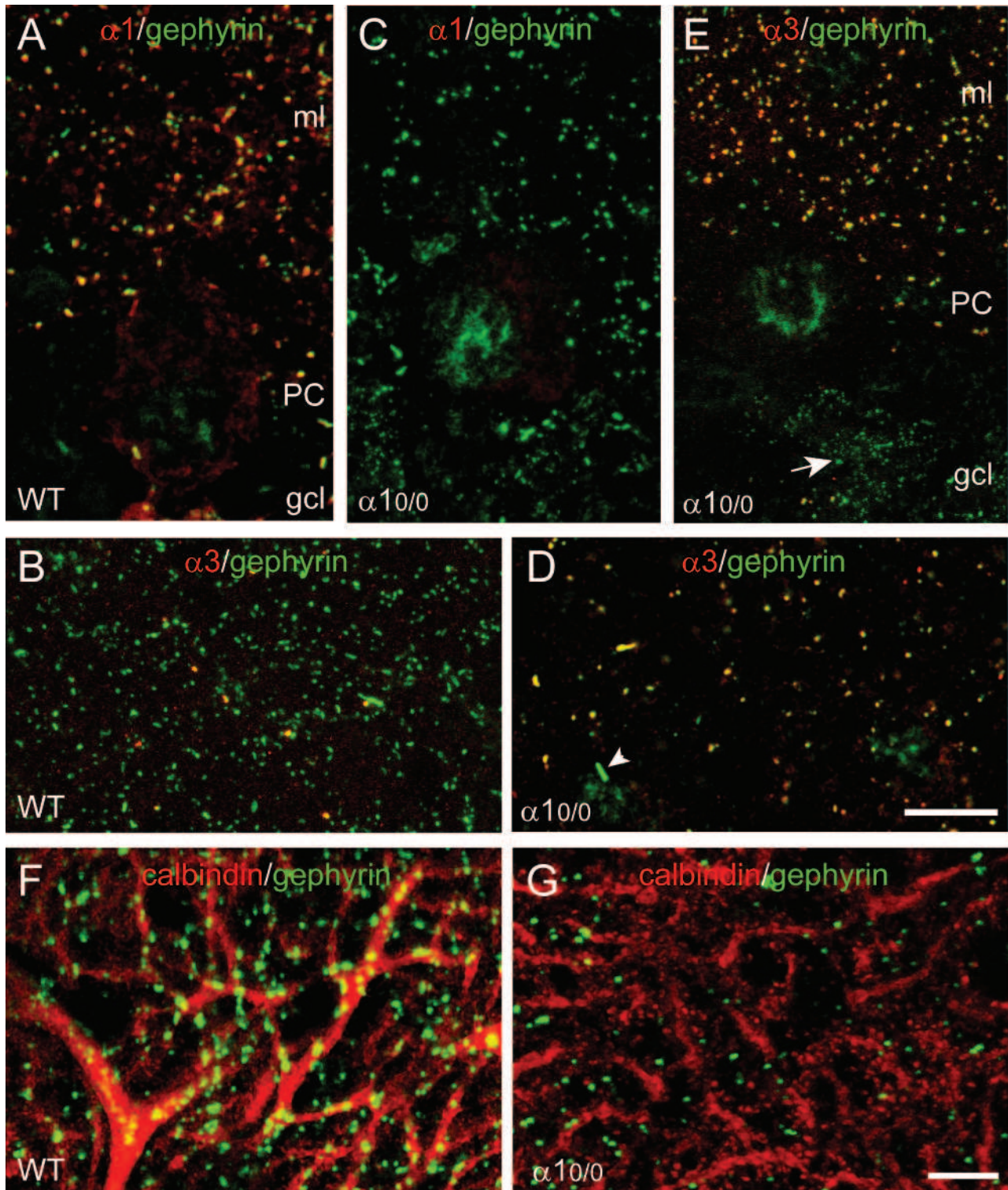


Figure 3. Partial disruption of gephyrin clustering and up-regulation of the $\alpha 3$ subunit in the molecular layer (ml) of $\alpha 1^{0/0}$ mice. Double immunofluorescence staining with the markers indicated in each panel. (A) Colocalization of the $\alpha 1$ subunit (red) and gephyrin (green) in discrete clusters (yellow) in the ml. Note that the soma of the PC is not labeled for gephyrin, whereas a few gephyrin clusters lacking the $\alpha 1$ subunit are evident in the ml. (B) Staining for the $\alpha 3$ subunit (red) in the ml reveals very few clusters, which are colocalized (yellow) with gephyrin (green). (C) Complete loss of $\alpha 1$ subunit staining and decreased density of gephyrin clusters (green) in the molecular layer of an $\alpha 1^{0/0}$ mouse. The weak labeling of the nucleus is non-specific and unrelated to the genotype. (D–E) Upregulation of $\alpha 3$ subunit-positive clusters (red) in the ml of $\alpha 1^{0/0}$ mice, which are associated with gephyrin (green) in virtually all remaining clusters. In panel D, the nuclei of two interneurons appear weakly stained in green. The arrowhead points to a large gephyrin aggregate, presumably located intracellularly in the interneuron. In panel E, note that no $\alpha 3$ subunit staining becomes apparent in the numerous small gephyrin clusters in the granule cell layer (gcl; arrow). (F–G) Loss of gephyrin clusters (green) on the dendrites of PC (labeled with calbindin, red) in $\alpha 1^{0/0}$ mice; the remaining gephyrin-positive clusters are mainly distributed on non-labeled structures between PC dendrites. Scale bars, A–D: 20 μm ; F–G: 10 μm . See (18) for details.

gephyrin is not due to a competitive disadvantage with $\alpha 1$ -GABA_A receptors. (ii) In PC expressing only the $\alpha 1$ -GABA_A receptors, no compensatory expression of another α subunit variant occurs. However, overexpression of other GABA_A receptor subtypes takes place in neighboring cells, such as Golgi and stellate cells, presumably to preserve the function of the cerebellar network. It is unclear how this up-regulation of $\alpha 3$ -GABA_A receptors is triggered, but stellate and Golgi cells are linked synaptically with PC via Lugaro cells (Figure 1). This observation strongly suggests that expression of GABA_A receptors is regulated by synaptic activity in the network. (iii) The upregulation of 'minor' GABA_A receptor subunits in the cerebellum of $\alpha 1^{0/0}$ mice occurs at the posttranslational level, as it is not accompanied by a change in mRNA transcription (81). Therefore, it appears that the turnover of GABA_A receptor subunits can be regulated dynamically, allowing neurons to adjust the use of specific GABA_A receptor subtypes for proper function.

The most likely implication of these changes is a reorganization of inhibitory circuits in the molecular layer of $\alpha 1^{0/0}$ mice to compensate for the loss of phasic and tonic inhibition in PCs. This conclusion, if confirmed by morphological analysis, implies that GABA_A receptors control, at least indirectly, the number of inhibitory synapses formed in a given circuit to set its overall level of activity. As shown in Figure 1, there are two major sources of inhibitory input on Golgi cells and molecular layer interneurons: Lugaro cells, which provide a dual glycinergic/GABAergic input onto Golgi cells and stellate/basket cells, which innervate each other and Golgi cells. This synaptic reorganization suggests growth of interneuron axonal arbors in the molecular layer of mutant mice and/or change in synaptic target specificity. A possible mechanism underlying this change in connectivity is discussed in the next section.

Requirement of GABA_A receptors for long-term maintenance of GABAergic synapses

The observation that PC in $\alpha 1^{0/0}$ mice are completely devoid of functional GABA_A receptors provided a unique incentive to investigate the effect of such a deletion on GABAergic synapses (9,86). At the light microscopy level, the morphology of PCs, as revealed by staining for calbindin or parvalbumin is unaffected in $\alpha 1^{0/0}$ mice and they are present in normal numbers (86). Likewise, the distribution of climbing fibers is undistinguishable from that seen in wildtype mice (9). GABAergic terminals in the molecular are readily detectable, but are larger and less regularly distributed than in wildtype. However, striking alterations in the organization of GABAergic synapses were evident at the ultrastructural level (9),

as summarized in Figure 4. In sections processed for GABA immunogold labeling, the vast majority of GABAergic presynaptic profiles formed synapses with PC spines, characterized by an asymmetric postsynaptic specialization indistinguishable from that formed in glutamatergic synapses (Figure 4A). These aberrant presynaptic terminals nevertheless were GABAergic, as shown by the presence of vesicular GABA transporter and the absence of vesicular glutamate transporters type 1 and type 2, which were readily detected in parallel (Figure 4C) and climbing fibers, respectively. Furthermore, most aberrant GABAergic terminals originated from stellate or basket cells, as seen by labeling for parvalbumin. On the dendrites of PC, the number of symmetric, type II synapses was reduced by about 75%, indicating a failure to form or to maintain GABAergic synapses in the absence of postsynaptic GABA_A receptors (9).

Unexpectedly, GABAergic synapses on PC somata, which are formed selectively by basket cell terminals, were not affected in $\alpha 1^{0/0}$ mice and retained their morphology, neurochemical phenotype, and content of presynaptic vesicles (Figure 4B). Therefore, the synaptic deficit observed in the molecular layer is selective for axo-dendritic synapses. Morphological and functional examination of the cerebellum during postnatal development revealed no transient expression of GABA_A receptors in PC of mutant mice. GABAergic axons contacting PC dendrites and somata were seen from P7 onwards. Ultrastructurally, the first synapses formed by terminals immunolabeled for GABA were symmetric on dendritic shafts. However, aberrant terminals, forming heterologous synapses with spines appeared first at P10 and their number increased rapidly thereafter (Figure 4D). These results were taken as evidence that initial steps of GABAergic synapse formation are not affected in $\alpha 1^{0/0}$ mice (9). However, a clear deficit for the maintenance of GABAergic synapses on PC dendrites occurs in the absence of functional GABAergic transmission.

Altogether, these observations confirm that neurotransmitter-mediated activity is not essential for synapse formation (91–94) and indicate that differential mechanisms ensure long-term stability of synapses in various cell compartments. While the normal morphology and content of presynaptic vesicles of basket cell terminals suggest that these terminals remain competent for GABA synthesis and release, activation of postsynaptic GABA_A receptors is not required for maintenance of this synapse. A different situation prevails in dendrites, where most GABAergic synapses are lost in adult animals. It is possible that spines exert a synaptogenic action of GABAergic terminals, which is sufficient to attract them when they form 'silent' contacts with their normal postsynaptic targets. It is

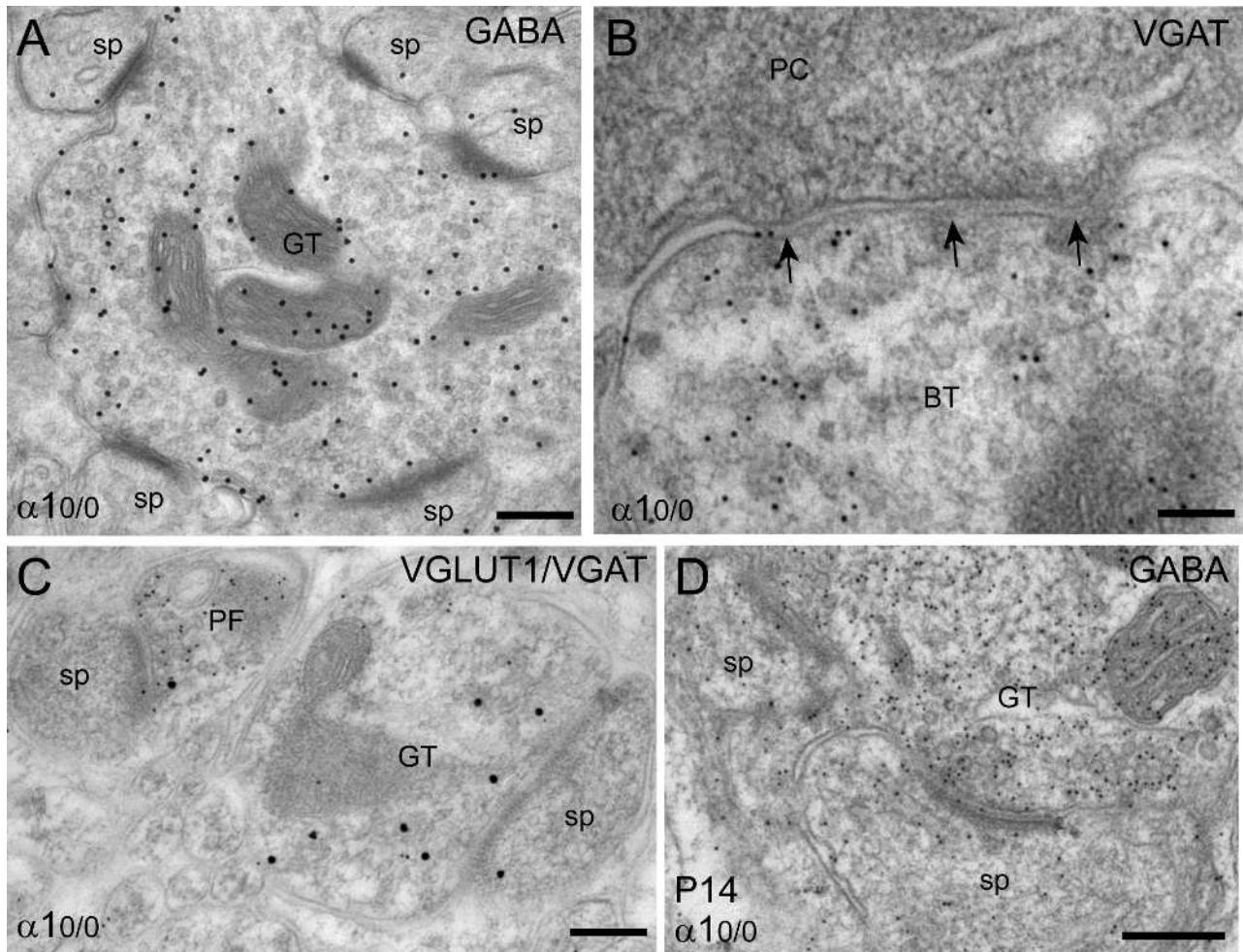


Figure 4. Formation of heterologous synapses in the molecular layer of $\alpha 1^{0/0}$ mice, as visualized by immunoelectron microscopy. Each panel depicts a section from a mutant mouse (A–C) adult, (D) P14. Immunogold labeling of mitochondria is non-specific and should be disregarded. (A) Example of a GABAergic terminal (GT) labeled for GABA using a secondary antibody coupled to 20 nm gold particles, which forms asymmetric synapses with 6 spine profiles (sp) within a single ultrathin section. (B) A basket cell terminal (BT) labeled for vesicular GABA transporter (VGAT; 10 nm immunogold particles) makes symmetric synapses (arrows) with the soma of a PC. Such terminals were indistinguishable from those seen in wildtype mice. (C) Segregation of type 1 vesicular glutamate transporter (VGLUT1; 10 nm gold particles) and VGAT (20 nm gold particles) between parallel fiber terminals (PF) and GABAergic terminals, even when both make asymmetric synapses with spines. (D) Example of heterologous synapses formed during postnatal development between a GABAergic terminal and two spines (GABA labeling with 10 nm gold particles). Scale bars, A, D: 200 nm; B, C: 100 nm. For details, see (9).

also conceivable that the presence of GABA_A receptors and/or gephyrin is required for synaptic anchoring of a molecule mediating transsynaptic homo- or heterophilic interaction with a presynaptic partner, thereby explaining the profound reduction of symmetric synapses on dendritic shafts. It is of note that the formation of basket cell synapses on the axon initial segment of PC depends on specific interactions with extracellular molecules (95) and that gephyrin clusters are not detectable on PC somata, despite the prominent clustering of the $\alpha 1$ subunit (Figure 3), indicating a distinct mechanism for GABA_A receptor clustering compared to dendrites. In any case, the lack of long-term stability of stellate cell synapses onto PC dendrites might contribute to the formation of supernumerary synapses between interneurons, as discussed in the previous section.

Conclusions

The analysis of targeted deletion of GABA_A receptor subunit genes expressed in the cerebellum has unraveled seemingly contradictory findings: GABA_A receptor subtypes are functionally not interchangeable and do not replace a missing subunit, as seen best by the loss of δ subunit protein in $\alpha 6^{0/0}$ mice and by the increase in extrasynaptic receptors in granule cells of $\alpha 1^{0/0}$ mice. However, wide ranging compensations contribute to the maintenance of function, in particular when the mutation concerns a major GABA_A receptor subtype. Adaptations can even occur in cell types that are not affected by the mutation, as witnessed by the increase in $\alpha 3$ -GABA_A receptors in Golgi cells of $\alpha 1^{0/0}$ mice. As a consequence, behavioral deficits are minimal compared to the strong impairment produced by acute pharmacological blockade or

stimulation of GABA_A receptors in vivo. While the stereotyped organization of the cerebellum facilitates these investigations, additional studies will be required to determine to which extent these findings can be generalized to other brain regions and other GABA_A receptor subtypes.

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References

- Barnard EA, Skolnick P, Olsen RW, Mohler H, Sieghart W, Biggio G, et al. International Union of Pharmacology. XV. Subtypes of γ -aminobutyric acid_A receptors: Classification on the basis of subunit structure and function. *Pharmacol Rev.* 1998;50:291–313.
- Fritschy JM, Brünig I. Formation and plasticity of GABAergic synapses: physiological mechanisms and pathophysiological implications. *Pharmacol Ther.* 2003;98:299–323.
- Sieghart W, Ernst M. Heterogeneity of GABA_A receptors: Revived interest in the development of subtype-selective drugs. *Curr Med Chem.* 2005;5:217–242.
- Rudolph U, Mohler H. GABA-based therapeutic approaches: GABA_A receptor subtype functions. *Curr Opin Pharmacol.* 2006;6:18–23.
- Poltl A, Hauer B, Fuchs K, Tretter V, Sieghart W. Subunit composition and quantitative importance of GABA_A receptor subtypes in the cerebellum of mouse and rat. *J Neurochem.* 2003;87:1444–1455.
- Wisden W, Korpi ER, Bahn S. The cerebellum: A model system for studying GABA_A receptor diversity. *Neuropharmacol.* 1996;35:1139–1160.
- Palay SL, Chan-Palay V. Cerebellar cortex: Cytology and organization. Berlin, Heidelberg, New York: Springer Verlag, 1974.
- Fritschy JM, Mohler H. GABA_A-receptor heterogeneity in the adult rat brain: Differential regional and cellular distribution of seven major subunits. *J Comp Neurol.* 1995;359:154–194.
- Fritschy JM, Panzanelli P, Kralic JE, Vogt KE, Sassoè-Pognetto M. Differential dependence of axo-dendritic and axo-somatic GABAergic synapses on GABA_A receptors containing the $\alpha 1$ subunit in Purkinje cells. *J Neurosci.* 2006;26:3245–3255.
- Riquelme R, Miralles CP, De Blas AL. Bergmann glia GABA_A receptors concentrate on the glial processes that wrap inhibitory synapses. *J Neurosci.* 2002;22:10720–10730.
- Muller T, Fritschy JM, Grosche J, Pratt GD, Mohler H, Kettenmann H. Developmental regulation of voltage-gated K⁺-channel and GABA_A-receptor expression in Bergmann glial cells. *J Neurosci.* 1994;14:2503–2514.
- Sassoè-Pognetto M, Panzanelli P, Sieghart W, Fritschy JM. Co-localization of multiple GABA_A receptor subtypes with gephyrin at postsynaptic sites. *J Comp Neurol.* 2000;420:481–498.
- Laurie DJ, Seeburg PH, Wisden W. The distribution of 13 GABA_A receptor subunit mRNAs in the rat brain. II. Olfactory bulb and cerebellum. *J Neurosci.* 1992;12:1063–1076.
- Wisden W, McNaughton LA, Darlison MG, Hunt SP, Barnard EA. Differential distribution of GABA_A receptor mRNAs in bovine cerebellum-localization of $\alpha 2$ mRNA in Bergmann glia layer. *Neurosci Lett.* 1989;106:7–12.
- Hevers W, Luddens H. Pharmacological heterogeneity of γ -aminobutyric acid receptors during development suggests distinct classes of rat cerebellar granule cells in situ. *Neuropharmacol.* 2002;42:34–47.
- Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G. GABA_A receptors: Immunocytochemical distribution of 13 subunits in the adult rat brain. *Neuroscience.* 2000;101:815–850.
- Mugnaini E, Floris A. The unipolar brush cell: a neglected neuron of the mammalian cerebral cortex. *J Comp Neurol.* 1994;339:174–180.
- Kralic JE, Sidler C, Parpan F, Homanics G, Morrow AL, Fritschy JM. Compensatory alteration of inhibitory synaptic circuits in thalamus and cerebellum of GABA_A receptor $\alpha 1$ subunit knockout mice. *J Comp Neurol.* 2006;495:408–421.
- Farrant M, Nusser Z. Variations on an inhibitory theme: Phasic and tonic activation of GABA_A receptors. *Nature Rev Neurosci.* 2005;6:215–229.
- Nusser Z, Sieghart W, Stephenson FA, Somogyi P. The $\alpha 6$ subunit of the GABA_A receptor is concentrated in both inhibitory and excitatory synapses on cerebellar granule cells. *J Neurosci.* 1996;16:103–114.
- Somogyi P, Fritschy JM, Benke D, Roberts JDB, Sieghart W. The $\gamma 2$ subunit of the GABA_A-receptor is concentrated in synaptic junctions containing the $\alpha 1$ and $\beta 2/3$ subunits in hippocampus, cerebellum and globus pallidus. *Neuropharmacol.* 1996;35:1425–1444.
- Nusser Z, Roberts JDB, Baude A, Richards JG, Somogyi P. Relative densities of synaptic and extrasynaptic GABA_A receptors on cerebellar granule cells as determined by a quantitative immunogold method. *J Neurosci.* 1995;15:2948–2960.
- Hausser M, Clark BA. Tonic synaptic inhibition modulates neuronal output pattern and spatiotemporal synaptic integration. *Neuron.* 1997;19:665–678.
- Konnerth A, Llano I, Armstrong CM. Synaptic currents in cerebellar Purkinje cells. *Proc Natl Acad Sci USA.* 1990;87:2662–2665.
- Zhang CL, Messing A, Chiu SY. Specific alteration of spontaneous GABAergic inhibition in cerebellar Purkinje cells in mice lacking the potassium channel Kv1.1. *J Neurosci.* 1999;19:2852–2864.
- Llano I, Gerschenfeld HM. Inhibitory synaptic currents in stellate cells of rat cerebellar slices. *J Physiol (Lond.).* 1993;468:177–200.
- Nusser Z, Cull-Candy S, Farrant M. Differences in synaptic GABA_A receptor number underlie variation in GABA mini amplitude. *Neuron.* 1997;19:697–709.
- Nusser Z, Sieghart W, Somogyi P. Segregation of different GABA_A receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. *J Neurosci.* 1998;18:1693–1703.
- Santhakumar V, Hancher HJ, Wallner M, Olsen RW, Otis TS. Contributions of the GABA_A receptor $\alpha 6$ subunit to phasic and tonic inhibition revealed by a naturally occurring polymorphism in the $\alpha 6$ gene. *J Neurosci.* 2006;26:3357–3364.
- Brickley SG, Cull-Candy S, Farrant M. Single-channel properties of synaptic and extrasynaptic GABA_A receptors suggest differential targeting of receptor subtypes. *J Neurosci.* 1999;19:2960–2973.
- Hevers W, Korpi ER, Luddens H. Assembly of functional $\alpha 6\beta 3\gamma 2\delta$ GABA_A receptors in vitro. *Neuroreport.* 2000;11:4103–4106.
- Saxena NC, Macdonald RL. Properties of putative cerebellar γ -aminobutyric acid_A receptor isoforms. *Mol Pharmacol.* 1996;49:567–579.

33. Rossi DJ, Hamann M, Attwell D. Multiple modes of GABAergic inhibition of rat cerebellar granule cells. *J Physiol.* 2003;548:97–110.
34. Rossi DJ, Hamann M. Spillover-mediated transmission at inhibitory synapses promoted by high affinity $\alpha 6$ subunit GABA_A receptors and glomerular geometry. *Neuron.* 1998;20:783–795.
35. Nusser Z, Mody I. Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. *J Neurophysiol.* 2002;87:2624–2628.
36. Cope DW, Hughes SW, Crunelli V. GABA_A receptor-mediated tonic inhibition in thalamic neurons. *J Neurosci.* 2005;25:11533–11563.
37. Stell BM, Brickley SG, Tang CY, Farrant M, Mody I. Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by δ subunit-containing GABA_A receptors. *Proc Natl Acad Sci USA.* 2003;100(24):14439–14444.
38. Dieudonne S. Glycinergic synaptic currents in Golgi cells of the rat cerebellum. *Proc Natl Acad Sci USA.* 1995;92:1441–1445.
39. Kaneda M, Farrant M, Cull-Candy SG. Whole-cell and single-channel currents activated by GABA and glycine in granule cells of the rat cerebellum. *J Physiol (Lond.).* 1995;485:419–435.
40. Aoki E, Semba R, Kashiwamata S. New candidates for GABAergic neurons in the rat cerebellum: an immunocytochemical study with anti-GABA antibody. *Neurosci Lett.* 1986;68:267–271.
41. Ottersen OP, Storm-Mathisen J, Somogyi P. Colocalization of glycine-like and GABA-like immunoreactivities in Golgi cell terminals in the rat cerebellum: A postembedding light and electron microscopic study. *Brain Res.* 1988;450:342–353.
42. Dumoulin A, Triller A, Dieudonne S. IPSC kinetics at identified GABAergic and mixed GABAergic and glycinergic synapses onto cerebellar Golgi cells. *J Neurosci.* 2001;21:6045–6057.
43. Dugue GP, Dumoulin A, Triller A, Dieudonne S. Target-dependent use of coreleased inhibitory transmitters at central synapses. *J Neurosci.* 2005;25:6490–6498.
44. Knuesel I, Mastrocola M, Zuellig RA, Bornhauser B, Schaub MC, Fritschy JM. Altered synaptic clustering of GABA_A-receptors in mice lacking dystrophin (*mdx* mice). *Eur J Neurosci.* 1999;11:4457–4462.
45. Knuesel I, Züllig RA, Bornhauser B, Schaub MC, Fritschy JM. Differential expression of utrophin and dystrophin in CNS neurons: An in situ hybridization and immunohistochemical study. *J Comp Neurol.* 2000;422:594–611.
46. Brünig I, Suter A, Knuesel I, Luscher B, Fritschy JM. GABAergic presynaptic terminals are required for postsynaptic clustering of dystrophin, but not of GABA_A receptors and gephyrin. *J Neurosci.* 2002;22:4805–4813.
47. Grady RM, Wozniak DF, Ohlemiller KK, Sanes JR. Cerebellar synaptic defects and abnormal motor behavior in mice lacking α - and β -dystrobrevin. *J Neurosci.* 2006;26:2841–2851.
48. Anderson JL, Head SI, Morley JW. Altered inhibitory input to Purkinje cells of dystrophin-deficient mice. *Brain Res.* 2003;982:280–283.
49. Anderson JL, Head SI, Morley JW. Long-term depression is reduced in cerebellar Purkinje cells of dystrophin-deficient *mdx* mice. *Brain Res.* 2004;1019:289–292.
50. Vaillend C, Billard JM. Facilitated CA1 hippocampal synaptic plasticity in dystrophin-deficient mice: role for GABA_A receptors? *Hippocampus.* 2002;12:713–717.
51. Vaillend C, Billard JM, Laroche S. Impaired long-term spatial and recognition memory and enhanced CA1 hippocampal LTP in the dystrophin-deficient DMD (*mdx*) mouse. *Neurobiol Disease.* 2004;17:10–20.
52. Wall MJ. Alterations in GABA_A receptor occupancy occur during the postnatal development of rat Purkinje cell but not granule cell synapses. *Neuropharmacol.* 2005;49:596–609.
53. Zempel JM, Steinbach JH. Neonatal rat cerebellar granule and Purkinje neurons in culture express different GABA_A receptors. *Eur J Neurosci.* 1995;7:1895–1905.
54. Gault LM, Siegel RE. Expression of the GABA_A receptor δ subunit is selectively modulated by depolarization in cultured rat cerebellar granule neurons. *J Neurosci.* 1997;17:2391–2399.
55. Behringer KA, Gault LM, Siegel RE. Differential regulation of GABA_A receptor subunit mRNAs in rat cerebellar granule neurons – importance of environmental cues. *J Neurochem.* 1996;66:1347–1353.
56. Zhu WJ, Vicini S, Harris BT, Grayson DR. NMDA-mediated modulation of γ -aminobutyric acid type A receptor function in cerebellar granule neurons. *J Neurosci.* 1995;15:7692–7701.
57. Thompson CL, Pollard S, Stephenson FA. Developmental regulation of expression of GABA_A receptor $\alpha 1$ and $\alpha 6$ subunits in cultured rat cerebellar granule cells. *Neuropharmacol.* 1996;35:1337–1346.
58. Takayama C, Inoue Y. Transient expression of GABA_A receptor $\alpha 2$ and $\alpha 3$ subunits in differentiating cerebellar neurons. *Dev Brain Res.* 2004;148:169–177.
59. Laurie DJ, Wisden W, Seeburg PH. The distribution of thirteen GABA_A receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. *J Neurosci.* 1992;12:4151–4172.
60. Gao B, Fritschy JM. Cerebellar granule cells in vitro recapitulate the in vivo pattern of GABA_A-receptor subunit expression. *Dev Brain Res.* 1995;88:1–16.
61. Bahn S, Harvey RJ, Darlison MG, Wisden W. Conservation of γ -aminobutyric acid type A receptor $\alpha 6$ subunit gene expression in cerebellar granule cells. *J Neurochem.* 1996;66:1810–1818.
62. Takayama C, Inoue Y. Morphological development and maturation of the GABAergic synapses in the mouse cerebellar layer. *Dev Brain Res.* 2004;150:177–190.
63. Mellor JR, Merlo D, Jones A, Wisden W, Randall AD. Mouse cerebellar granule cell differentiation: electrical activity regulates the GABA_A receptor $\alpha 6$ subunit gene. *J Neurosci.* 1998;18:2822–2833.
64. Farrant M, Brickley SG. Properties of GABA_A receptor-mediated transmission at newly formed Golgi-granule cell synapses in the cerebellum. *Neuropharmacol.* 2003;44:181–189.
65. Hevers W, Luddens H. Pharmacological heterogeneity of γ -aminobutyric acid receptors during development suggests distinct classes of rat cerebellar granule cells in situ. *Neuropharmacol.* 2002;42:34–47.
66. Paysan J, Fritschy JM. GABA_A-receptor subtypes in developing brain: Actors or spectators? *Perspect Dev Neurobiol.* 1998;5:179–192.
67. Fritschy JM, Paysan J, Enna A, Mohler H. Switch in the expression of rat GABA_A-receptor subtypes during postnatal development: an immunohistochemical study. *J Neurosci.* 1994;14:5302–5324.
68. Sinkkonen ST, Mihalek RM, Homanics GE, Luddens H, Korpi ER. Altered atypical coupling of γ -aminobutyrate type A receptor agonist and convulsant binding sites in subunit-deficient mouse lines. *Mol Brain Res.* 2001;86:179–183.
69. Makela R, Uusi-Oukari M, Homanics GE, Quinlan JJ, Firestone LL, Wisden W, et al. Cerebellar γ -aminobutyric acid type A receptors: Pharmacological subtypes revealed by mutant mouse lines. *Mol Pharmacol.* 1997;52:380–388.
70. Gunther U, Benson J, Benke D, Fritschy JM, Reyes GH, Knoflach F, et al. Benzodiazepine-insensitive mice generated by targeted disruption of the $\gamma 2$ -subunit gene of γ -aminobutyric acid type A receptors. *Proc Natl Acad Sci USA.* 1995;92:7749–7753.

71. Essrich C, Lorez M, Benson JA, Fritschy JM, Luscher B. Postsynaptic clustering of major GABA_A receptor subtypes requires the $\gamma 2$ subunit and gephyrin. *Nature Neurosci.* 1998;1:563–571.
72. Li RW, Yu W, Christie SB, Miralles CP, Bai J, Loturco JJ, et al. Disruption of postsynaptic GABA receptor clusters leads to decreased GABAergic innervation of pyramidal neurons. *J Neurochem.* 2005;95:756–770.
73. Nusser Z, Ahmad Z, Tretter V, Fuchs K, Wisden W, Sieghart W, et al. Alterations in the expression of GABA_A receptor subunits in cerebellar granule cells after the disruption of the $\alpha 6$ subunit gene. *Eur J Neurosci.* 1999;11:1685–1697.
74. Jones A, Korpi ER, McKernan RM, Pelz R, Nusser Z, Makela R, et al. Ligand-gated ion channel subunit partnerships: GABA_A receptor $\alpha 6$ subunit gene inactivation inhibits δ subunit expression. *J Neurosci.* 1997;17:1350–1362.
75. Uusi-Oukari M, Heikkilä J, Sinkkonen ST, Mäkelä R, Hauer B, Homanics GE, et al. Long-range interactions in neuronal gene expression: evidence from gene targeting in the GABA_A receptor $\beta 2$ - $\alpha 6$ - $\alpha 1$ - $\gamma 2$ subunit gene cluster. *Mol Cell Neurosci.* 2000;16:34–41.
76. Korpi ER, Koikkalainen P, Vekovischeva OY, Mäkelä R, Kleinz R, Uusi-Oukari M, et al. Cerebellar granule-cell-specific GABA_A receptors attenuate benzodiazepine-induced ataxia: evidence from $\alpha 6$ -subunit-deficient mice. *Eur J Neurosci.* 1998;11:233–240.
77. Tretter V, Hauer B, Nusser Z, Mihalek RM, Hoyer H, Homanics GE, et al. Targeted disruption of the GABA_A receptor δ subunit gene leads to an up-regulation of $\gamma 2$ subunit-containing receptors in cerebellar granule cells. *J Biol Chem.* 2001;276:10532–10538.
78. Vicini S, Losi G, Homanics GE. GABA_A receptor δ subunit deletion prevents neurosteroid modulation of inhibitory synaptic currents in cerebellar neurons. *Neuropharmacol.* 2002;43:646–650.
79. Fodor L, Biro T, Maksay G. Nanomolar allopregnanolone potentiates rat cerebellar GABA_A receptors. *Neurosci Lett.* 2005;383:127–130.
80. Brickley SG, Revilla V, Cull-Candy SG, Wisden W, Farrant M. Adaptive regulation of neuronal excitability by a voltage-independent potassium conductance. *Nature.* 2001;409:88–92.
81. Ogris W, Lehner R, Fuchs K, Furtmüller B, Hoyer H, Homanics GE, et al. Investigation of the abundance and subunit composition of GABA_A receptor subtypes in the cerebellum of $\alpha 1$ -subunit-deficient mice. *J Neurochem.* 2006;96:136–147.
82. Kralic JE, O'Buckley TK, Khisti RT, Hodge CJ, Homanics GE, Morrow AL. GABA_A receptor $\alpha 1$ subunit deletion alters receptor subtype assembly, pharmacological and behavioral responses to benzodiazepines and zolpidem. *Neuropharmacol.* 2002;43:685–694.
83. Kralic JE, Wheeler M, Renzi K, Ferguson C, O'Buckley TK, Grobin AC, et al. Deletion of GABA_A receptor $\alpha 1$ subunit-containing receptors alters responses to ethanol and other anesthetics. *J Pharmacol Exp Ther.* 2003;305:600–607.
84. Kralic JE, Korpi ER, O'Buckley TK, Homanics GE, Morrow A. Molecular and pharmacological characterization of GABA_A receptor $\alpha 1$ subunit knockout mice. *J Pharmacol Exp Ther.* 2002;302:1037–1045.
85. Sur C, Wafford KA, Reynolds DS, Hadingham KL, Bromidge F, Macaulay A, et al. Loss of the major GABA_A receptor subtype in the brain is not lethal in mice. *J Neurosci.* 2001;21:3409–3418.
86. Kralic JE, Criswell HE, Ostermann JL, O'Buckley TK, Wilkie ME, Matthews DA, et al. Genetic essential tremor in γ -aminobutyric acid_A receptor $\alpha 1$ subunit knockout mice. *J Clin Invest.* 2005;115:774–779.
87. Ortinski PI, Lu C, Takagaki K, Fu Z, Vicini S. Expression of distinct α subunits of GABA_A receptor regulates inhibitory synaptic strength. *J Neurophysiol.* 2004;92:1718–1727.
88. Vicini S, Ferguson C, Prybylowski K, Kralic JE, Morrow AL, Homanics GE. GABA_A receptor $\alpha 1$ subunit deletion prevents developmental changes of inhibitory synaptic currents in cerebellar neurons. *J Neurosci.* 2001;21:3009–3016.
89. Bosman LWJ, Heinen K, Spijker S, Brussaard AB. Mice lacking the major adult GABA_A receptor subtype have normal number of synapses, but retain juvenile IPSC kinetics until adulthood. *J Neurophysiol.* 2005;94:338–346.
90. Barberis A, Lu C, Vicini S, Mozrzymas JW. Developmental changes of GABA synaptic transient in cerebellar granule cells. *Mol Pharmacol.* 2005;67:1221–1228.
91. Verhage M, Maia AS, Plomp JJ, Brussaard AB, Heeroma JH, Vermeer H, et al. Synaptic assembly of the brain in the absence of neurotransmitter secretion. *Science.* 2000;287:864–869.
92. Varoqueaux F, Sigler A, Rhee JS, Brose N, Enk C, Reim K, et al. Total arrest of spontaneous and evoked synaptic transmission but normal synaptogenesis in the absence of Munc13-mediated vesicle priming. *Proc Natl Acad Sci USA.* 2002;99:9037–9042.
93. Gally C, Bessereau JL. GABA is dispensable for the formation of junctional GABA receptor clusters in *Caenorhabditis elegans*. *J Neurosci.* 2003;23:2591–2599.
94. Harms KJ, Craig AM. Synapse composition and organization following chronic activity blockade in cultured hippocampal neurons. *J Comp Neurol.* 2005;490:72–84.
95. Ango F, Di Cristo G, Higashiyama H, Bennett V, Wu P, Huang ZJ. Ankyrin-based subcellular gradient of neurofascin, an immunoglobulin family protein, directs GABAergic innervation at Purkinje axon initial segment. *Cell.* 2004;119:257–272.